

WHAT IS CLAIMED IS:

1. A method of quantifying the possible presence
of a target analyte molecularly recognizable in a speci-
5 men, the method comprising the following steps:

(a) subjecting a device to said target analyte,
said device having a membrane including a spot portion
that is chemically treated such that presence of said
target analyte can be made to change a characteristic of
10 said spot portion to permit contrast of said spot portion
relative to surrounding regions of said membrane to quan-
tify presence of said target analyte;

(b) alternately and periodically illuminating said
spot portion and said surrounding regions of said mem-
15 brane;

(c) measuring said characteristic of light
reflected by said spot portion and by said surrounding
regions, and comparing said characteristic light so
measured; and

20 (d) outputting a signal comparing said
characteristic of light so measured.

2. The method of claim 1, wherein said
characteristic includes at least one characteristic
25 selected from a group consisting of (i) color, (ii) color
density, (iii) optical density, and (iv) relative
contrast of reflected light.

30 3. The method of claim 1, wherein said target
analyte includes at least one analyte selected from a
group consisting of (i) nucleic acids, (ii) antigens,

(iii) antibodies, (iv) haptens, (v) hapten conjugates, (vi) macro-molecules, (vii) proteins, (viii) polymers, and (ix) chemicals.

5 4. The method of claim 1, wherein step (b) includes providing illumination from at least one light source selected from a group consisting of (i) a source of visible light, (ii) a source of non-visible light, (iii) a LED, (iv) a laser diode, (v) a source of 10 incandescent light, (vi) a source of X-rays, (vii) a source of ultra-violet, (viii) a source of infra-red, (ix) a source of diffuse light, (x) a source of non-diffuse light.

15 5. The method of claim 1, wherein step (b) includes at least one step selected from a group consisting of (i) illuminating said spot portion with a circular light pattern and illuminating said surrounding regions with annular light patterns, (ii) illuminating 20 said spot portion with a circular light pattern and illuminating at least one of said surrounding regions with a circular light pattern, (iii) illuminating at least one of said spot portion and said surrounding regions with a non-circular light pattern, (iv) providing said step of illuminating from multiple light sources, (v) providing said step of illuminating from a single light source, (vi) alternately and periodically illuminating with a frequency ranging from about 100 Hz to about 10 KHz, and (vii) alternately and periodically 25 switching illumination from said spot portion to said surrounding regions.

6. The method of claim 1, wherein:

step (b) includes illuminating at least one of said spot portion and said surrounding regions with a circular light pattern, wherein said spot portion and said surrounding regions lie on a line; and

5 step (c) includes disposing light detectors, to carry out said measuring, on a line normal to said line such that said spot portion and said surrounding regions are each substantially equidistant from said light
10 detectors.

7. The method of claim 1, wherein step (b) includes providing a single source of light and alternately and periodically illuminating by selectively passing light from said source of light through a component selected from a group consisting of (i) a rotatable disk defining through openings placed and sized to pass light directed to at least one chosen region of said membrane, (ii) an electronic shutter defining regions selectively made transparent to pass light directed to at least one chosen region of said membrane, and (iii) a liquid crystal shutter defining regions selectively made transparent to pass light directed to at least one chosen region of said membrane.

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8. The method of claim 1, wherein step (b) includes measuring intensity with at least first and second light detectors spaced-apart from each other a multiple of 90° azimuthal such that skew errors from
30 irregularities in topography of said membrane are reduced.

9. The method of claim 1, wherein step (c) includes subtracting measured light intensity from said spot region from measured light intensity of said surrounding regions, said subtracting being carried out
5 in a manner selected from a group consisting of (i) analog subtraction, and (ii) digital subtraction;

wherein effects of measured intensity in absence of light are reduced.

10 10. The method of claim 1, wherein step (d) includes at outputting at least one signal selected from a group consisting of (i) a signal representing a ratio of measured reflected light from said spot portion to measured reflected light from said surrounding regions,
15 (ii) providing at least first and second light detectors and outputting a signal representing a ratio of measured reflected light from said spot portion to measured intensity of illumination of said spot portion, (iii) providing at least first and second light detectors and
20 outputting a signal representing a ratio of measured reflected light from said surrounding portions to measured intensity of illumination of said surrounding portions, (iv) a digital reading signal, (v) an analog reading signal, (vi) providing at least first and second
25 light detectors and outputting a signal representing a ratio of ratios, the first of which ratios is represented by measured reflect light from said spot portion compared to measured intensity of illumination of said portion, and a second of which ratios is represented by a ratio of
30 measured reflected light from said surrounding portions to measured intensity of illumination of said surrounding

portions, (vii) a light that changes color proportional to said signal, (viii) a light that changes intensity proportional to said signal, (ix) a printed output, (x) a digital computer-interfaceable signal, (xi) a sound whose 5 pitch is proportional to said signal, (xii) a sound whose amplitude is proportional to said signal, and (xiii) an audible enunciator enunciating at least one word appropriate to said signal.

10 11. The method of claim 1, wherein:
 step (b) includes alternately and periodically illuminating with a chosen frequency in a range of about 100 Hz to about 10 KHz; wherein
 step (d) includes at least two steps selected from a 15 group consisting of:

 measuring reflected light intensity, representing measured light intensity reflected by said spot portion and measured light intensity reflected by said surrounding regions, synchronously with illuminating in 20 step (a);

 combining inverted and non-inverted measured reflected light intensity representing measured light intensity reflected by said spot portion and measured light intensity reflected by said surrounding regions;

25 synchronously switchably sampling signals representing an amplified version of the inverted and non-inverted measured reflected light intensities to provide said output signal.

30 12. The method of claim 11, wherein step (d) further includes at least one step selected from a group

consisting of (i) filtering measured said light intensity to reduce frequency components associated with said chosen frequency, and (ii) low-pass filtering the synchronously switchably sampled signals before providing
5 said output signal.

13. A reflectometry system for measuring intensity of a spot on a substrate relative to intensity of a surrounding area of said substrate, the system including:
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a master clock unit, outputting at least a periodic master clock signal having a clock frequency and duty cycle;

15 at least one light source, coupled to an output of said master clock unit, emitting light controllably directed at said spot, and emitting light controllably directed at said surrounding area;

first and second light detectors disposed as to detect fractions of said light reflected by said surrounding area and by said spot;

20 wherein output signals from said first and second light detectors include said quantifying data.

14. The system of claim 13, wherein said first and second light detectors are spaced-apart a azimuthal angular amount from each other such that skew errors from irregularities in topography of said substrate are reduced, said angular amount being selected from a group consisting of (i) 180°, and (ii) 90°.
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30 15. The system of claim 13, further including means for sampling and detecting output signals from said first

and second light detectors, synchronously with said periodic master clock signal, to provide said quantifying data.

5 16. The system of claim 13, further including:
means for substantially cancelling response of said first and second light detectors to zero light input and for substantially cancelling drift in said output signals from said first and second light detectors; said means
10 for substantially cancelling outputting said quantifying data.

15 17. The system of claim 16, wherein said means for substantially cancelling includes a lock-in amplifier system.

20 18. The system of claim 16, further including:
a summing amplifier coupled to said output signals from said first and second light detectors to output an average summed signal;
a non-inverting amplifier and an inverting amplifier, each said amplifier have equal gain and being AC-coupled to receive said average summed signal from said summing amplifier;
25 a switch coupled to alternatively sample, synchronously with said master clock signal, an output of said non-inverting amplifier and an output of said inverting amplifier, such sampling occurring during a transition-free interval of an active portion of said master clock signal duty cycle during which said light source is driven;

wherein an output of said switch includes an average component representing said quantifying data.

19. The system of claim 13, wherein said at least
5 one light source illuminates said substrate in at least
one manner selected from a group consisting of (i) said
spot and said surrounding area are alternately
illuminated, (ii) said spot and said surrounding area are
simultaneously illuminated, and (iii) only said spot is
10 illuminated and alternatively said surrounding area but
for said spot is illuminated.

20. The system of claim 13, wherein said light
source is selected from a group consisting of (i) a
15 source of visible light, (ii) a source of non-visible
light, (iii) a LED, (iv) a laser diode, (v) a source of
incandescent light, (vi) a source of X-rays, (vii) a
source of ultra-violet, (viii) a source of infra-red,
(ix) a source of diffuse light, (x) a source of non-
20 diffuse light.